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Effect of inoculum source and initial concentration on the anaerobic digestion of the liquid fraction from hydrothermal carbonisation of sewage sludge

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Abstract

Hydrothermal carbonisation (HTC) is a relatively new alternative for the management of sewage sludge that allows obtaining a HTC char (hydrochar) with a high heating value (≈22 MJ/kg). The aim of this work has been to study the anaerobic digestion of the liquid fraction generated as by-product during HTC (LFHTC) of dewatered sewage sludge, to get more value to the overall process. For this purpose, three different inocula: granular biomass from industrial reactors treating brewery and sugar beet wastewaters and a flocculent biomass from a full-scale digester of municipal sewage sludge at two initial inoculum concentrations (IC) (10 and 25 g COD/L) were tested. ANOVA test was applied to evaluate the ultimate methane yield for each IC. The effect was different for each inoculum studied: an increase from 10 to 25 g COD/L increased the methane yield by 23% for brewery waste, achieving the highest value obtained (177±5 mL STP $CH_4/gCOD_{added}$), while declining to 99±2 mL STP $CH_4/gCOD_{added}$ for sugar beet; it is not affected by the municipal sludge, yielding around 135 mL STP $CH_4/gCOD_{added}$. Therefore, among the inocula tested, brewery waste was the most appropriate for the anaerobic digestion of the LFHTC of dewatered sewage sludge at high IC.

Keywords

Biochemical methane potential (BMP), dewatered sewage sludge, hydrothermal carbonisation (HTC), initial inoculum concentration (IC).

1. Introduction

Currently, farmland utilisation, incineration and landfilling are the main methods for sewage sludge biosolid (stabilised sewage sludge) disposal (Fytili and Zabaniotou, 2008). In the last decades, thermal valorisation of sewage sludge, including pyrolysis and gasification, has been gaining attention (Alvárez et al., 2015; Kokalj et al., 2017). Hydrothermal carbonisation (HTC) is a thermochemical process for converting organic feedstock with high moisture into a carbon-rich solid product (HTC char or hydrochar) with a higher heating value compared to the biochar produced from conventional carbonisation at similar temperatures (Kambo and Duta, 2015; Koottatep et al., 2016, Popov et al., 2016). This thermal process is performed under relatively low temperatures (180–375 °C) and auto-generated pressure for variable lengths (Pham et al., 2015), turning out to be an attractive option for dewatered sewage sludge (DSS) valorisation. The product from HTC of biomass is a slurry that can be separated into a solid and a liquid fraction. This liquid fraction (LFHTC) contains at least 15% of the initial carbon content (Broch et al., 2014) and can be used as potential source of chemicals or fuels through the biorefinery concept (Xiao et al., 2012). It can also be subjected to aerobic degradation (Eibisch et al., 2013; Ramke et al., 2009), wet air oxidation (Reza et al., 2016) or anaerobic digestion (Becker et al., 2014; Danso-Boateng et al., 2015; de la Rubia et al., 2018; Erdogan et al., 2015; Oliveira et al., 2013; Qiao et al., 2011; Smith and Ross, 2016; Villamil et al., 2018; Weiner et al., 2016; Wirth et al., 2015; Wood et al., 2013), the last being a potential route of valorisation towards biogas production.

To establish the interest of using a given feedstock for anaerobic digestion, batch tests can be performed to determine the methane potential, i.e., the maximum methane quantity that can be potentially produced. To optimise anaerobic digestion, different parameters related to the substrate, the inoculum and the operating conditions need to be checked, with the inoculum source and the inoculum to substrate ratio (ISR) being the most relevant (Elbeshbishy et al., 2012; Pellera and Gidarakos, 2016; Raposo et al., 2011).

The inoculum determines the initial activity of the microorganisms used for the test. Therefore, successful start-up and operation of anaerobic reactors require a seed sludge with a well-balanced microbial community (Oz et al., 2012; Steinmetz et al., 2016). During the process, it is expected that the microbial communities can adapt because of the growth of microorganisms under the specific digestion conditions and the substrate treatment (Rincón et al., 2011). Different inocula sources have been checked, including sludge from anaerobic digesters treating municipal or agro-industrial wastewater, animal manures and landfill leachate, among others (Córdoba et al., 2016; Foster-Carneiro et al., 2007; Moreno-Andrade and Buitrón, 2004; Pereira et al., 2002; Rincón et al., 2011; Tabatabaei et al., 2010).

Selection of the ISR is a determining issue for correct operation of anaerobic digestion. It depends largely on the substrate used, considering the potential amount of nitrogen ammonia and volatile fatty acids (VFA) produced from the hydrolysis of total Kjeldahl nitrogen (TKN) and the acidification of the organic matter, respectively (Elbeshbishy et al., 2012). Values of approximately 2 have been usually recommended (Alzate et al., 2012; Pellera and Gidarakos, 2016; Raposo et al., 2011, Villamil et al., 2018), although

76 in some cases, lower values have been successfully used (Pozdniakova et al., 2012;
77 Raposo et al., 2006; Sri Bala Kameswari et al., 2012).
78
79 The aim of this work is to study the valorisation of the by-product (liquid fraction)
80 generated from HTC of secondary dewatered sewage sludge. The hydrothermal
81 carbonisation could be a very suitable process to transform dewatered sewage sludge into
82 a peat-like material without pre-drying the biomass input, in a new concept for sewage
83 management. The energetic balance is more favourable than for thermal alternative
84 processes, converting biomass as a whole (Garlapalli et al., 2016; Kambo and Dutta,
85 2015; Lucian and Fiori, 2017; Stemann et al., 2013). With the purpose of completing the
86 process, the liquid fraction from HTC could be treated with the raw wastewater or co-
87 digested with the primary sewage sludge. The potential to produce methane of this liquid
88 by-product separated from the hydrochar has been scarcely studied so far. Hence, a more
89 in-deep study of the anaerobic digestion of the HTC by-product is required in order to
90 evaluate the best conditions for optimizing methane yield. Batch tests have been carried
91 out to determine the ultimate methane yield and the evolution of key parameters during
92 the anaerobic digestion process, evaluating the influence of the inoculum source and their
93 concentration. Thus, three inocula sources were compared: a flocculent sludge from an
94 anaerobic digester treating the sewage sludge of a municipal wastewater treatment plant
95 (MWTP) and two granular anaerobic sludges from a mesophilic upflow anaerobic sludge
96 blanket (UASB) reactor treating effluents from sugar beets and from a mesophilic internal
97 circulation reactor treating brewery wastewater. Two inoculum concentrations (IC) (10
98 and 25 g COD/L) were tested for each inoculum, keeping constant the ISR at 2 (on the
99 basis of COD).
100

2. Materials and Methods

2.1 HTC process

HTC was performed at 208 °C in a 4 L ZipperClave® pressure vessel electrically heated using 1.5 kg of DSS (85% moisture) which was collected from a full-scale membrane bioreactor treating industrial wastewaters from a cosmetics factory. The operating temperature was reached at a heating rate of 3 °C/min and maintained for 1 h. The reaction was stopped by cooling with an internal heat exchanger using tap water. The slurry obtained (470 g of wet hydrochar and 530 g of LFHTC for each kg of wet material treated) was centrifuged (1400g for 1 h) by a SIGMA 3-16L centrifuge equipped with a fixed angle rotor (cod. 12159). The liquid fraction was recovered by filtration (0.45 μm) and was maintained at 4 °C to be used as substrate of the anaerobic digestion tests performed.

2.2 Substrate and inocula seed for anaerobic experiments

The main characteristics and composition of the LFHTC were as follows (given as average values of three determinations with standard deviations): pH: 5.1±0.1; soluble COD (SCOD): 95.5±0.4 g/L; biochemical oxygen demand (BOD_5): 25.6±1.1 g/L; total solids (TS): 55.7±0.5 g/L, volatile solids (VS): 46.2±0.5 g/L; total organic carbon (TOC): 42.6±0.9 g/L; and TKN: 8.7±0.1 g/L. The substrate was analysed by GC/MS and HPLC/RI. These analyses allowed identification of the presence of nitrogen-containing species (pyrazines and aromatic amines) and oxygenated aromatic compounds (phenols and furans) and determination of the VFA concentration, respectively.

Three different inocula, collected from industrial full-scale anaerobic reactors operating under mesophilic conditions (35 °C), were used for the anaerobic digestion batch experiments:

- Inoculum 1 (BW): Granular inoculum obtained from an internal circulation anaerobic reactor treating brewery wastewater.
- Inoculum 2 (SB): Granular anaerobic sludge from an UASB reactor treating sugar beet effluents.
- Inoculum 3 (MS): Flocculent anaerobic sludge from a sewage sludge digester of a MWTP.

The main characteristics of the three inocula are collected in Table 1 (average values of three determinations with standard deviations). Substantial differences can be observed due to the substrate treatment and the reactor configuration of the full-scale facilities.

2.3 Batch anaerobic experiments

Anaerobic digestion runs were carried out batch-wise in 120 mL glass serum vials, filled with 60 mL of a suspension of inoculum, substrate and a basal medium with macro- and micronutrients, as described elsewhere (Rincón et al., 2011) following the recommendations of Holliger et al. (2016). The reaction medium was previously flushed with N_2 for 3 min in order to achieve anaerobic conditions. Then, the vials were sealed with rubber stoppers and metallic crimps. The vials were maintained in a static incubator at mesophilic temperature (35±1 °C) and manually mixed on a daily basis. As indicated before, two IC values (10 and 25 g COD/L) were tested with each inoculum. Taking into account the above cited literature and previous studies developed with LFHTC (Villamil et al., 2018), an ISR of 2 (on the basis of COD) was chosen for experiments performing.

149 This ISR corresponds to 2.6 for BW and MS inocula and to 2.2 for SB one on a VS basis.
150 All the experiments were run for approximately 40-45 days until no significant gas
151 production was observed or less than 5% of the total produced (on the last day) (Holliger
152 et al., 2016).
153
154 Triplicate blank samples with no substrate were run to determine the background methane
155 from the inocula and triplicate control experiments with starch (Panreac) were also
156 conducted with each inoculum to verify their activity, and yields higher than 85% of
157 theoretical (350 mL $CH_4 \cdot g^{-1}$ COD_{added}) were reached for the three inocula tried. For both
158 IC tested with every inoculum, 9 batch runs were carried out. Six of them were sacrificed
159 every one or two days initially and then weekly to follow the time-course of the anaerobic
160 digestion process. The other three runs were used only for biogas measurements (volume
161 and composition).
162
163 *2.4 Analytical methods*
164 The inocula were characterised by measuring the pH (using a model Crison 20 Basic pH
165 meter), TS and VS according to the standard methods 2540B and 2540E (APHA, 1998).
166 The total COD (TCOD) was determined following the method proposed by Raposo et al.
167 (2008). TKN determination has been described elsewhere (Villamil et al., 2018).
168
169 The liquid fraction from hydrothermal carbonisation, as well as the sacrificed samples
170 (centrifuged and filtered through a 0.45 μm filter), were used to determine the following
171 parameters: pH; partial and total alkalinity (PA and TA) by pH titration to 5.75 and 4.3,
172 respectively (Jenkins et al., 1983); intermediate alkalinity (IA), defined as the difference
173 between TA and PA; SCOD, using the closed digestion and colorimetric standard method

5220D (APHA, 1998); TOC, measured with an automatic analyser TOC-VCPN (Shimadzu); TKN; and TAN, determined by distillation and titration according to the standard method 4500E (APHA, 1998). Analyses of individual VFAs (C2-C4) were performed by HPLC/IR (Varian, Agilent Technologies, Santa Clara, CA, USA) (Rajhi et al., 2016). Identification of individual compounds from LFHTC was carried out by GC–MS (CP-3800/Saturn 2200 using a Varian CP-8200 autosampler injector) (De la Rubia et al., 2018). The compounds were assessed using the NIST 2008 Library.

Biogas and methane production were measured daily during the first 3 days and eight more times for the rest of the incubation period. Biogas production was determined by a manometric method (Rozzi and Remigi, 2004), measuring the pressure increase in each vial by an electronic pressure monitor (ifm, PN 7097). It was expressed at standard temperature and pressure (STP: 273 K, 1 bar). Biogas was subsequently exhausted to re-establish atmospheric pressure. The gas composition (H_2, CO_2 and CH_4) was determined by gas chromatography using a Bruker 450-GC (Goes, The Netherlands) coupled with a thermal conductivity detector (TCD) for H_2 and CO_2 and a flame ionisation detector (FID) for CH_4 (Rajhi et al., 2016). Methane production was calculated by subtracting the amount of methane produced in the blank controls from the methane production of each batch reactor.

2.5. Statistical analyses

Methane yields were expressed as mean value ± standard deviation (average of three samples). Analysis of variance (ANOVA) was carried out using Origin software (version 9.0). The significant of means values was determined by Fisher's test. Fisher's least significant difference (Fisher's LSD) was calculated at a confidence level of 0.05.

199

200

201 **3. Results and discussion**

202 Figure 1a shows the time-course of the pH during the anaerobic digestion of the LFHTC
203 of sewage sludge. The initial pH for all the inocula ranged between 7 and 7.8 and
204 increased during the anaerobic process to 7.5-8.1. As can be seen, this parameter is more
205 affected for the IC, with higher values for experiments carried out at IC 2.5 and final
206 values varying less than 0.1, than for the type of inoculum. Anyway, these pH values are
207 compatible with the adequate growth of anaerobic microorganisms, including
208 methanogenic *Archaea* (Franke-Whittle et al., 2014). The evolution of alkalinity (Figure
209 1b) shows a fairly similar trend at each IC value, regardless of the inoculum source. In all
210 cases, the alkalinity increased during the first 8-10 days and then remained almost
211 constant for the rest of the experiment. In the IC 1 runs, alkalinity values of 1900-2100
212 mg $CaCO_3$/L were reached, while the values for IC 2.5 experiments ranged between 4000
213 and 5400 mg $CaCO_3$/L. The increase of alkalinity, clearer for IC 2.5 runs, must be due to
214 the release of ammonia nitrogen and carbon dioxide upon the decomposition of the
215 organic matter. This favours the buffer capacity of the system, as has been previously
216 reported (Córdoba et al, 2016). The expected relationship between pH and alkalinity
217 appears clearly.

218

219 The evolution of TAN (Figure 1c) showed similar trends with the three inocula as those
220 observed for pH and alkalinity. Percentages above 70% of TAN were released at the 8^{th}
221 day in all the cases, except for SB 2.5 run, which confirm the extent of the hydrolytic
222 stage until that time. Values above 1700 mg N/L could inhibit the biogas yield and

promote high VFA concentrations (Franke-Whittle, 2014), although it was not achieved for any conditions assessed, only SB 2.5 run was close to this value.

The lower initial alkalinity for the experiments with MS inoculum (compared with BW and SB ones) affected the pH of the corresponding runs, especially during the first 5 days (hydrolytic-acidogenic stage), related also to the increase of TVFA concentration (Figure 2a). Figure 2 shows the time-course of TVFA (2a) and SCOD (2b), respectively. These parameters provide useful information on the performance of the anaerobic digestion process relative to the acidogenesis level (TVFA evolution) and the degradability of the substrate (SCOD reduction). In the case of TVFA, values in the range of 200-1000 mg COD/L were observed at IC 1 runs during the hydrolytic-acidogenic stage (first 5-8 d), while the final concentration were almost negligible for every experiment. Therefore, no intermediate products in the form of VFA were accumulated. These results are consistent with the trend observed for SCOD removal and with the IA/TA ratios that were maintained within adequate levels, between 0.19 and 0.29. However, certain amounts of SCOD remained at the end of the assays. This fraction of the non-removed COD can be associated with oxygen- and nitrogen-bearing aromatic compounds, generated during the carbonisation of the sewage sludge and identified in the initial LFHTC (Villamil et al., 2018). As a representative example, Figure 3 depicts the GC/MS chromatograms of the initial and final samples from the SB at IC 1 experiment. Table 2 collects the assessed compounds and the corresponding removal percentages in the liquid phase from the IC 1 runs with the three inocula used. Anaerobic digestion led to almost complete removal of the ketone (benzophenone) and aldehyde (4-methoxycinnamaldehyde) species present in the initial samples. Phenols and other oxygenated aromatics were partially removed, but a new phenolic compound (phenol, 2,4-bis(1,1-dimethylethyl)-) appeared as a

248 degradation intermediate. The starting LFHTC showed a high concentration of TKN,
249 probably due to the presence of nitrogen-containing species such as pyrazines and
250 aromatic amines (p-aminotoluene; pyrazine, 2,5-dimethyl; pyrazine, 2-ethyl-5-methyl-;
251 pyrimidine, 4,6-dimethyl-; benzenamine, 3-methyl-; 4,5-dimethyl-ortho-
252 phenylenediamine), which showed different resistances to anaerobic degradation. This
253 fact was more evident in the experiments carried out with BW inoculum, which upheld
254 the highest final SCOD values (Figure 2b), with removal efficiencies between 58 and
255 65%, while for SB and MS inocula removal efficiencies ranged between 78 and 90%. The
256 different removal pattern of the organic compounds from the liquid phase could be related
257 to biodegradation and/ or to sorption processes on the sludge, being the latest relevant in
258 the removal of a number of organic compounds (toxicant/ inhibitors) previously to
259 anaerobic biodegradation (Chen et al., 2014).
260
261 *Methane yield*
262 Incubation with different inocula resulted in dissimilar methane yields (mL STP CH_4/g
263 COD_{added}), as seen in Figure 4. SB and MS achieved the highest methane yields for the
264 IC 1 runs, with 138±18 and 127±13 mL STP CH_4/g COD_{added}, respectively. Meanwhile,
265 with BW, the highest methane yield was obtained for the IC 2.5 run, with 177±5 mL STP
266 CH_4/g COD_{added}. The BW granular sludge gave the highest methane yields. Neves et al.
267 (2004) and Rincón et al. (2011) reported better results with granular than with flocculent
268 sludge for kitchen wastes and sunflower oil cake, respectively. De Vrieze et al. (2015)
269 also recommended the use of granular sludge as inoculum because of its higher
270 methanogenic abundance and diversity, which determines a higher activity compared
271 with other kinds of inocula. The low yield reached with SB granular inoculum could be
272 explained by its poor granulation. In addition, this inoculum has the highest TKN values,

releasing the highest concentration of TAN (see Figure 1c), the obtained value ≈1500 mg N/L is close to the considered as inhibitory as has been commented above. Additionally, SB presented the highest concentration of TS but the lowest percentage of VS (23.2%); therefore, the expected activity should be lower. In this way, the methane yield was 9 and 35% higher in the 1 and 2.5 experiments, respectively, with MS and 14 and 79.5% higher with BW. Variation of the IC, which resulted in modifications of substrate concentration at a constant ISR, showed significantly different effects on the methane productivity, depending on the inoculum. Increasing the IC from 10 to 25 g COD/L improved the methane yield by 23% with the BW inoculum but decreased it by 22% with the SB one. However, the IC within the range tested did not affect the methane production rate with the MS inoculum, after an observed lag period. Significant differences of maximum methane yield were found for BW 2.5 run ($p < 0.05$), while the differences of this parameter were not significant ($p > 0.05$) for SB 1, BW 1, MS 1 and MS 2.5 experiments (Table 3). According to Table 3, SB 2.5 showed significantly lower methane yields than the other types of inocula or inoculum concentrations. The percentage of methane in the biogas was related to the inoculum, reaching up to 82, 73 and 62% with BW, MS and SB, respectively.

Wirth et al. (2015) obtained similar methane yields to those of the current work with the liquid fraction from HTC of digested sewage sludge operating in continuous feed mode (120-180 mL CH_4 STP/g COD_{added}). Meanwhile, Qiao et al. (2011) reported 256.7 mL CH_4/g COD with the LFHTC of mixed sewage sludge. The anaerobic digestion of the LFHTC of several residues has been studied recently. The ultimate methane yields are affected by the raw residue nature and moisture and the carbonisation conditions (temperature and time). Values in the range of 175-300 mL CH_4/g COD have been

298 reached with the LFHTC of stillage (Wood et al., 2013), orange pomace (Erdogan et al.,
299 2015), chaff (Weiner et al., 2016), and a mixture of polysaccharides, proteins, and lipids
300 representing food waste (Posmanik et al., 2017).
301
302 The results of methane yield from the experiments with the SB and BW inocula were
303 fitted to a first-order kinetic model (Eq. 1) frequently applied to methane production from
304 anaerobic digestion experiments (Elbeshbishy et al., 2012; Fernandez-Cegri et al., 2012;
305 Pellera and Gidarakos, 2016; Posmanik et al., 2017; Raposo et al., 2011; Rodriguez-
306 Chiang and Dahl, 2015; Wang et al., 2015). The Gompertz model (Eq. 2) has been used
307 for the MS experiments due to the initial lag-phase observed with this inoculum (De la
308 Rubia et al., 2018; Park et al., 2017; Pellera and Gidarakos, 2016; Pozdniakova et al.,
309 2012; Ran et al., 2018; Shen et al., 2018) :
310 $G = Gm \cdot [1 - \exp(-k \cdot t)]$ Eq. (1)
311 $G = Gm \cdot [1 - \exp(-k \cdot (t-\lambda)]$ Eq. (2)
312
313 where G represents the cumulative methane yield at time t; G_m is the ultimate methane
314 yield of the substrate tested, i.e., the final value when no more gas is released from the
315 reactor; k is the specific rate or apparent kinetic constant; and λ is the extent of the lag-
316 phase (d). Origin software (version 8.0) was used to fit the experimental data to equations
317 (1) and (2). Table 3 summarises the k values with 95% confidence, as well as the
318 corresponding values of Gm and R^2. The high values of the coefficient of determination
319 R^2 (> 0.96) and the low values of the confidence limits of the parameters show the good
320 fit of the experimental data to the proposed models. An initial lag-phase was observed
321 with MS inoculum, which could be related with their flocculent structure. In fact, an initial
322 hydrolysis (cellular lysis) determined by the increase of SCOD was observed only for this

inoculum (Figure 2b), showing a very low methane yield until an acclimation period of 5-7 days. Interestingly, after that period, methane rate production with MS inoculum was considerably higher than the obtained with SB and BW inocula. Actually, the highest *k* values were obtained for MS inoculum (≈0.29 d^{-1}) while significantly lowest values were reached for BW (≈0.04 d^{-1}), that reveals a slower digestion.

Conclusions

The results obtained in this study reveal the importance of the inoculum origin and structure for the treatment of the LFHTC of sewage sludge by anaerobic digestion. IC increase can improve the methane yield depending on the inoculum source. High COD removal efficiencies were achieved for each inocula studied and the methane yields were very dependent of the inoculum source. Among the three inocula tested, BW appears to be the best in terms of methane production, although the significantly lowest values of the kinetic constant reveal a slower digestion. Further research will be required to evaluate the co-digestion of this liquid by-product with primary sludge in order to integrate the HTC of sewage sludge in wastewater treatment plants.

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349 **References**

350 Alvarez, J., Amutio, M., Lopez, G., Barbarias, I., Bilbao, J., Olazar, M. 2015. Sewage
351 sludge valorization by flash pyrolysis in a conical spouted bed reactor. Chem. Eng.
352 J. 273, 173-183.

353 American Public Health Association/American Water Works Association/Water
354 Environment Federation. Standard Methods for the Examination of Water and
355 Wastewater, 20th edn. 1998. Washington, DC.

356 Alzate, M.E., Muñoz, R., Rogalla, F., Fdz-Polanco, F., Pérez-Elvira, S.I. 2012.
357 Biochemical methane potential of microalgae: Influence of substrate to inoculum
358 ratio, biomass concentration and pretreatment. Bioresour. Technol. 123, 488–494.

359 Becker, R., Dorgerloh, U., Paulke, E., Mumme, J., Nehls, I. 2014. Hydrothermal
360 carbonization of biomass: major organic components of the aqueous phase. Chem.
361 Eng. Technol. 37, 511–518.

362 Broch, A., Jena, U., Hoekman, S.K., Langford, J. 2014. Analysis of solid and aqueous
363 phase products from hydrothermal carbonization of whole and lipid-extracted algae.
364 Energies 7, 62-79.

365 Chen, J.L., Ortiz, R., Steele, T.W.J., Stuckey, D.C. 2014. Toxicants inhibiting anaerobic
366 digestion: A review. Biotechnol. Adv. 32, 1523–1534.

367 Córdoba, V., Fernández, M., Santalla, E. 2016. The effect of different inoculums on
368 anaerobic digestion of swine wastewater. J. Env. Chem. Eng. 4, 115-122.

369 Danso-Boateng, E., Shama, G., Wheatley, A.D., Martin, S.J., Holdich, R.G. 2015.
370 Hydrothermal carbonisation of sewage sludge: Effect of process conditions on
371 product characteristics and methane production. Bioresour. Technol. 177, 318–327.

372 De la Rubia, M.A., Villamil, J.A., Rodríguez, J.J., Borja, R., Mohedano, A.F., 2018.
373 Mesophilic anaerobic co-digestion of the organic fraction of municipal solid waste
374 with the liquid fraction from hydrothermal carbonization of sewage sludge. Waste
375 Manage., DOI 10.1016/j.wasman.2018.02.046.

376 De Vrieze, J., Raport, L., Willems, B., Verbrugge, S., Volcke, E., Meers, E., Angenent,
377 L.T., Boon, N. 2015. Inoculum selection influences the biochemical methane
378 potential of agro-industrial substrates. Microbial Biotechnol. 8, 776–786.

379 Eibisch, N., Helfrich, M., Don, A., Mikutta, R., Kruse, A., Ellerbrock, R., Flessa, H. 2013.
380 Properties and degradability of hydrothermal carbonization products. J. Environ.
381 Qual. 42, 1565–1573.

382 Elbeshbishy, E., Nakhla, G., Hafez, H. 2012. Biochemical methane potential (BMP) of
383 food waste and primary sludge: Influence of inoculum pre-incubation and inoculum
384 source. Bioresour. Technol. 110, 18–25.

385 Erdogan, E., Atila, B., Mumme, J., Reza, M.T., Toptas, A., Elibol, M., Yanik, J. 2015.
386 Characterization of products from hydrothermal carbonization of orange pomace
387 including anaerobic digestibility of process liquor. Bioresour. Technol. 196, 35–42.

388 Fernández-Cegrí, V., De la Rubia, M.A., Raposo, F., Borja, R. 2012. Effect of
389 hydrothermal pretreatment of sunflower oil cake on biomethane potential focusing
390 on fibre composition. Bioresour. Technol. 123, 424–429.

391 Fytili, D., Zabaniotou, A. 2008. Utilization of sewage sludge in EU application of old and
392 new methods-A review. Renew. Sust. Energ. Rev. 12, 116-140.

393 Foster-Carneiro, T., Pérez, M., Romero, L.I., Sales, D. 2007. Dry-thermophilic anaerobic
394 digestion of organic fraction of the municipal solid waste: focusing on the inoculum
395 sources. Bioresour. Technol. 98, 3195-3203.

396 Franke-Whittle, I.H., Walter, A., Ebner, C., Insam, H. 2014. Investigation into the effect
397 of high concentrations of volatile fatty acids in anaerobic digestion on methanogenic
398 communities. Waste Manage. 34, 2080–2089.
399 Garlapalli, R.K., Wirth, B., Reza, M.T. 2016. Pyrolysis of hydrochar from digestate:
400 Effect of hydrothermal carbonization and pyrolysis temperatures on pyrochar
401 formation. Bioresour. Technol. 220, 168–174.
402 Holliger, C., Alves, M., Andrade, D., Angelidaki, I. et al. 2016. Towards a standardization
403 of biomethane potential tests. Wat. Sci. Technol. 74, 2515-2522.
404 Jenkins, S., Morgan, J., Sawyer, C. 1983. Measuring anaerobic sludge digestion and
405 growth by a simple alkalimetric titration, Water Pollut. Control Fed. 55, 448–453.
406 Kambo, H.S., Dutta, A. 2015. A comparative review of biochar and hydrochar in terms
407 of production, physico-chemical properties and applications. Renew. Sust. Energ.
408 Rev. 45, 359–378.
409 Kokalj, F., Arbiter, B., Samec, N., 2017. Sewage sludge gasification as an alternative
410 energy storage model. Energ. Convers. Manage. in press. DOI:
411 10.1016/j.enconman.2017.02.076.
412 Koottatep, T., Fakkaew, K., Tajai, N., Pradeep, S.V., Polprasert, C. 2016. Sludge
413 stabilization and energy recovery by hydrothermal carbonization process. Renew.
414 Energy 99, 978-985.
415 Lucian, M., Fiori, L. 2017. Hydrothermal Carbonization of Waste Biomass: Process
416 Design, Modelling, Energy Efficiency and Cost Analysis. Energies 10, 211.
417 Moreno-Andrade, I., Buitrón, G. 2004. Influence of the origin of the inoculum on the
418 anaerobic biodegradability test. Water Sci. Technol. 49, 53-59.

419 Neves, L., Oliveira, R., Alves, M.M. 2004. Influence of inoculum activity on the bio-
420 methanization of a kitchen waste under different waste/inoculum ratios. Process
421 Biochem. 39, 2019–2024.
422 Oliveira, I., Blöhse, D., Ramke, H-G. 2013. Hydrothermal carbonization of agricultural
423 residues. Bioresour. Technol. 142, 138–146.
424 Oz, N.A., Ince, O., Turker, G., Ince, B.K. 2012. Effect of seed sludge microbial
425 community and activity on the performance of anaerobic reactors during the start-up
426 period. World J. Microbiol Biotechnol. 28, 637–647.
427 Park, S., Yoon, Y.-M., Han, S.K., Kim, D., Kim, H. 2017. Effect of hydrothermal pre-
428 treatment (HTP) on poultry slaughterhouse waste (PSW) sludge for the enhancement
429 of the solubilization, physical properties, and biogas production through anaerobic
430 digestion. Waste Manage. 64, 327-332.
431 Pellera, F-M., Gidarakos, E. 2016. Effect of substrate to inoculum ratio and inoculum
432 type on the biochemical methane potential of solid agroindustrial waste. J. Env.
433 Chem. Eng. 4, 3217–3229.
434 Pereira, M.A., Mota, M., Alves, M.M. 2002. Operation of an anaerobic filter and an
435 EGSB reactor for the treatment of an oleic acid-based effluent: influence of inoculum
436 quality. Process Biochem. 37, 1025-1031.
437 Pham, T.P.T., Kaushik, R., Parshetti, G.K., Mahmood, R., Balasubramanian, R. 2015.
438 Food waste-to-energy conversion technologies: Current status and future directions.
439 Waste Manage. 38, 399–408.
440 Popov, S., Abdel-Fattah, T., Kumar, S. 2016. Hydrothermal treatment for enhancing oil
441 extraction and hydrochar production from oilseeds. Renew. Energy 85, 844-853.

442 Posmanik, R., Labatut, R.A., Kim, A.H., Usack, J.G., Tester, J.W., Angenent, L.A. 2017.
443 Coupling hydrothermal liquefaction and anaerobic digestion for energy valorization
444 from model biomass feedstocks. Bioresour. Technol. 233, 134–143.
445 Pozdniakova, T.A., Costa, J.C., Santos, R.J., Alves, M.M., Boaventura, R.A.R. 2012.
446 Anaerobic biodegradability of Category 2 animal by-products: Methane potential and
447 inoculum source. Bioresour. Technol. 124, 276–282.
448 Qiao, W., Peng, C., Wang, W., Zhang, Z. 2011. Biogas production from supernatant of
449 hydrothermally treated municipal sludge by upflow anaerobic sludge blanket reactor.
450 Bioresour. Technol. 102, 9904–9911.
451 Rajhi, H., Puyol, D., Martínez, M.C., Díaz, E.E, Sanz, J.L. 2016. Vacuum promotes
452 metabolic shifts and increases biogenic hydrogen production in dark fermentation
453 systems. Front. Environ. Sci. Eng. 10, 513–521.
454 Ramke, H.G., Blöhse, D., Lehmann, H.J., Fettig, J., 2009. Hydrothermal Carbonization
455 of Organic Waste in: Cossu, R.; Diaz, L. F.; Stegmann, R. (Edts.), 2009: Sardinia
456 2009: Twelfth International Waste Management and Landfill Symposium, Sardinia,
457 Italy, 05-09 October 2009, Proceedings, CISA Publisher.
458 Ran, G., Li, D., Zheng, T., Liu, X., Chen, L., Cao, Q., Yan, Z., 2018. Hydrothermal
459 pretreatment on the anaerobic digestion of washed vinegar residue. Bioresour.
460 Technol. 248, 265–271.
461 Raposo, F., Banks, C.J., Siegert, I., Heaven, S., Borja, R. 2006. Influence of inoculum to
462 substrate ratio on the biochemical methane potential of maize in batch tests. Process
463 Biochem. 41, 1444-1450.
464 Raposo, F., de la Rubia, M.A., Borja, R. Alaiz, M. 2008. Assessment of a modified and
465 optimised method for determining chemical oxygen demand of solid substrates and
466 solutions with high suspended solid content. Talanta 76, 448–453.

467 Raposo, F., Fernandez-Cegri, V., De la Rubia, M.A., Borja, R., Beline, F., et al. 2011.
468 Biochemical methane potential (BMP) of solid organic substrates: evaluation of
469 anaerobic biodegradability using data from an international interlaboratory study. J.
470 Chem. Technol. Biotechnol. 86, 1088–1098.
471 Reza, M.T., Freitas, A., Yang, X., Coronella, C.J. 2016. Wet air oxidation of
472 hydrothermal carbonization (HTC) process liquid. ACS Sustainable Chem. Eng. 4,
473 3250−3254.
474 Rincón, B., Portillo, M.C., González, J.M., Fernández-Cegrí, V., De la Rubia, M.A.,
475 Borja, R. 2011. Feasibility of sunflower oil cake degradation with three different
476 anaerobic consortia. J. Environ. Sci. Heal. A 46, 1409-1416.
477 Rodriguez-Chiang, L.M., Dahl, O.P. 2015. Effect of inoculum to substrate ratio on the
478 methane potential of microcrystalline cellulose production wastewater.
479 BioResources 10, 898-911.
480 Rozzi, A., Remigi, E. 2004. Methods of assessing microbial activity and inhibition under
481 anaerobic conditions: a literature review. Rev. Environ. Sci. Biotechnol. 3, 93–115.
482 Shen, J., Yan, H., Zhang, R., Liu, G., Chen, C. 2018. Characterization and methane
483 production of different nut residue wastes in anaerobic digestion. Renew. Energy
484 116, 835-841.
485 Smith, A.M., Ross, A.B. 2016. Production of bio-coal, bio-methane and fertilizer from
486 seaweed via hydrothermal carbonisation. Algal Res. 16, 1–11.
487 Sri Bala Kameswari, K., Kalyanaraman, C., Porselvam, S., Thanasekaran, K. 2012.
488 Optimization of inoculum to substrate ratio for bio-energy generation in codigestión
489 of tannery solid wastes. Clean Technol. Envir. 14, 241–250.
490 Steinmetz, R.L.R., Mezzari, M.P., da Silva, M.L.B., Kunz, A., do Amaral, A.C., Tápparo,
491 D.C., Soares, H.M. 2016. Enrichment and acclimation of an anaerobic mesophilic

492 microorganism's inoculum for standardization of BMP assays. Bioresour. Technol.
493 219, 21-28.
494 Stemann, J., Erlach, B., Ziegler, F. 2013. Hydrothermal Carbonisation of Empty Palm Oil
495 Fruit Bunches: Laboratory Trials, Plant Simulation, Carbon Avoidance, and
496 Economic Feasibility. Waste Biomass Valori. 4, 441–454.
497 Tabatabaei, M., Rahim, R.A., Abdullah, N., Wright, A.G., Shirai, Y., Sakai, K., Sulaiman,
498 A., Hassan, M.A. 2010. Importance of the methanogenic archaea populations in
499 anaerobic wastewater treatments. Process Biochem. 45, 1214-1225.
500 Villamil, J.A., De la Rubia, M.A., Mohedano, A., Rodriguez, J.J. 2018. Valorisation of
501 the liquid fraction from hydrothermal carbonisation of sewage sludge by anaerobic
502 digestion. J. Chem. Technol. Biotechnol. 98, 450-456.
503 Wang, B., Strömberg, S., Li, C., Nges, I.A., Nistor, M., Deng, L., Liu, J. 2015. Effects of
504 substrate concentration on methane potential and degradation kinetics in batch
505 anaerobic digestion. Bioresour. Technol. 194, 240–246.
506 Weiner, B., Wedwitschka, H., Poerschmann, J., Kopinke, F-D. 2016. Utilization of
507 organosolv waste waters as liquid phase for hydrothermal carbonization of chaff.
508 Sustainable Chem. Eng. 4, 5737−5742.
509 Wirth, B., Reza, T., Mumme, J. 2015. Influence of digestion temperature and organic
510 loading rate on the continuous anaerobic treatment of process liquor from
511 hydrothermal carbonization of sewage sludge. Bioresour. Technol. 198, 215–222.
512 Wood, B.M., Jader, L.R, Schendel, F.J., Hahn, N.J., Valentas, K.J., McNamara, P.J.,
513 Novak, P.M., Heilmann, S.M. 2013. Industrial symbiosis: corn ethanol fermentation,
514 hydrothermal carbonization, and anaerobic digestion. Biotechnol. Bioeng. 110,
515 2624-2632.

516 Xiao, L.P., Shi, Z.J., Xu, F., Sun, R.C. 2012. Hydrothermal carbonization of
517 lignocellulosic biomass. Bioresour. Technol. 118, 619-623.

Table 1. Main characteristics of the seed inocula.

Inoculum source	**pH**	**TS (g/kg)**	**VS (g/kg)**	**CODt (g/L)**	**TKN (g N/L)**
BW	7.6±0.1	61.9±0.9	55.7±0.9	91.2±1.4	2.2±0.1
SB	7.9±0.1	162.4±4.0	37.8±0.6	73.4±0.1	4.4±0.1
MS	7.2±0.1	43.3±0.3	26.5±0.3	43.5±1.2	2.2±0.1

Table 2. Removal/generation of chemical species upon anaerobic digestion of the liquid fraction from hydrothermal carbonisation of sewage sludge for samples at IC 1 and every inoculum.

Compound	**Retention time (min)**	**Peak number**	**Removal (%)***		
			BW	MS	SB
Aldehydes					
4-Methoxycinnamaldehyde	12.9	14	>99	97	87
Nitrogenated compounds					
p-Aminotoluene	3.4	1	n.d.	0	0
Pyrazine, 2,5-dimethyl	3.5	2	0	23	88
Pyrimidine, 4,6-dimethyl-	3.6	3	0	8	81
Benzenamine, 3-methyl-	3.8	4	3	0	27
Pyrazine, 2-ethyl-5-methyl-	4.9	5	0	32	61
4,5-Dimethyl-ortho-phenylenediamine	6.4	7	0	9	37
Oxygenated aromatics					
4-Isopropylcyclohexanone	5.7	6	n.d	38	78
7H-Dibenzo(a,g)carbazole, 12,13-dihydro-	7.3	8	0	0	0
Benzene, 1,2,4,5-tetramethyl-	7.6	9	0	40	39
Phenol, 2,3,5,6-tetramethyl-	7.8	10	13	42	93
Phenol, 2-methyl-6-(2-propenyl)-	9.2	11	>99	34	44
Benzene, 1-methoxy-4-(1-propenyl)-	10.5	12	20	18	23
Phenol, p-tert-butyl-	11.8	13	85	62	100
Phenol, 2,4-bis(1,1-dimethylethyl)-	14.5	15	gen	gen	gen
Benzophenone	16.6	16	99	99	87
1,2,3,4,5,6-Hexahydro-1,1,5,5-tetramethyl-2,4a-methanonaphthalen	18.7	17	n.d.	n.d	>99
1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	20.0	18	0	0	13

*with respect to peak area

nd: not detected

gen: generated

Table 3. Values of the apparent kinetic constant (k), lag phase (λ) and fitted (Gm) and experimental (Gm_e) maximum methane yield.

Experiment	**k** (d^{-1})	**λ** (d)	Gm (mL CH_4/g COD_{added})	R^2	Gm_e (mL CH_4/g COD_{added})
SB 1	0.196±0.016	-	121.6±3.3	0.974	$127±13^b$
SB 2.5	0.198±0.012	-	91.0±1.8	0.987	$99±1^c$
BW 1	0.043±0.009	-	165.3±17.2	0.966	$144±1^b$
BW 2.5	0.034±0.006	-	228.4±24.2	0.983	$177±5^a$
MS 1	0.302±0.047	5.19±0.39	124.0±4.9	0.969	$138±8^b$
MS 2.5	0.281±0.027	5.35±0.25	126.4±3.2	0.988	$133±11^b$

Means with different superscript significant differ ($p < 0.05$)

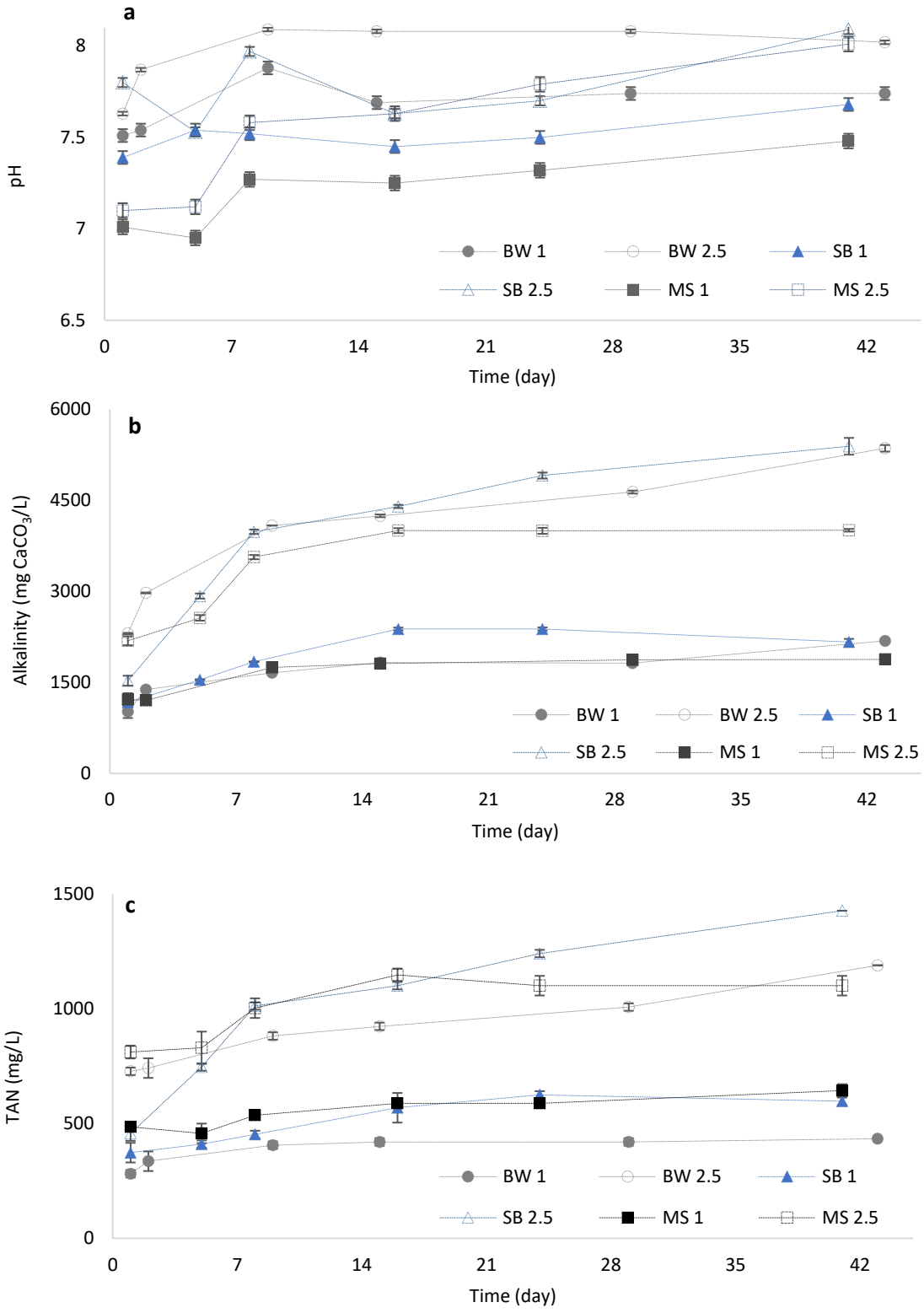

Figure 1. Time-course of pH (a), alkalinity (b) and TAN (c) during the anaerobic digestion of the LFHTC of sewage sludge.

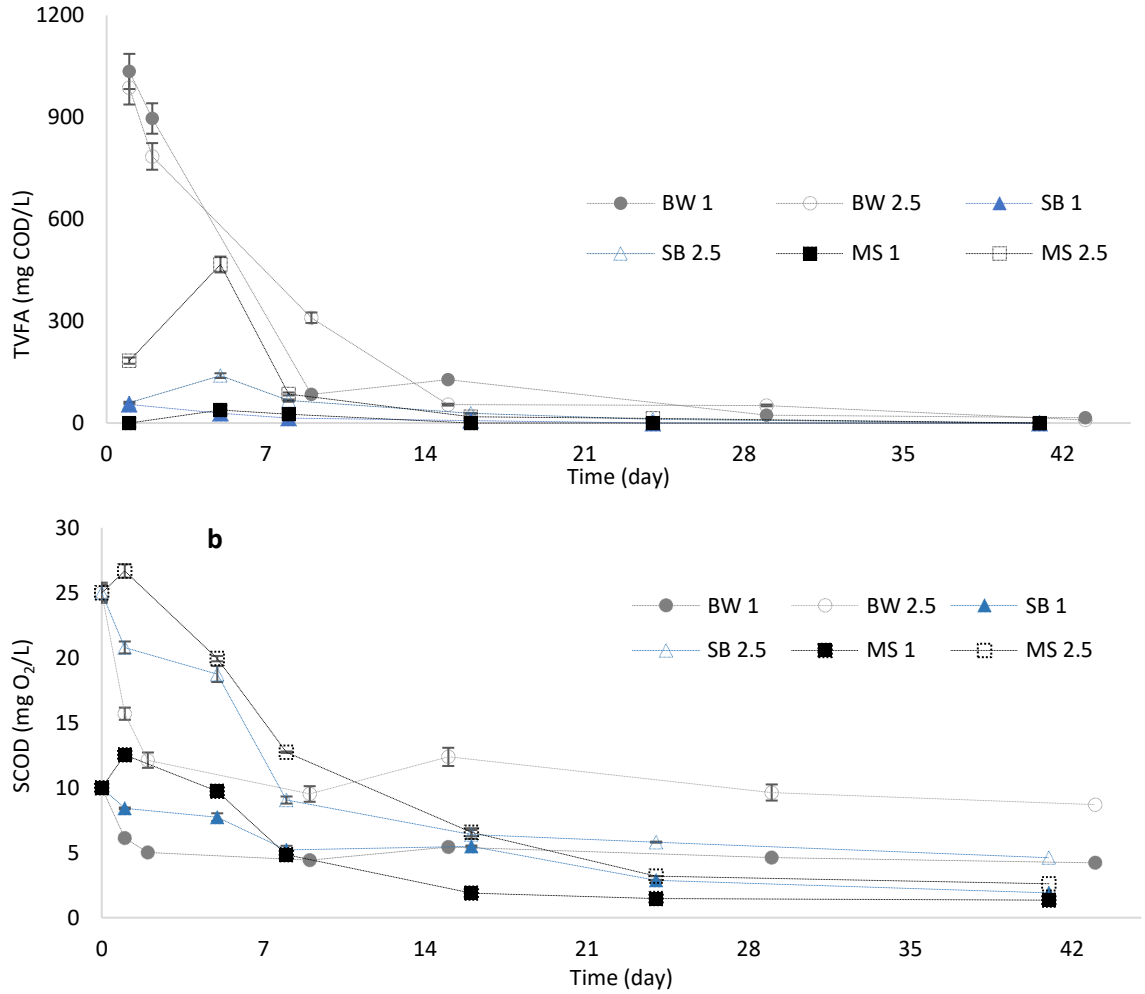

Figure 2. Time-course of total volatile fatty acids (a) and soluble chemical oxygen demand (b) during the anaerobic digestion of the LFHTC of sewage sludge

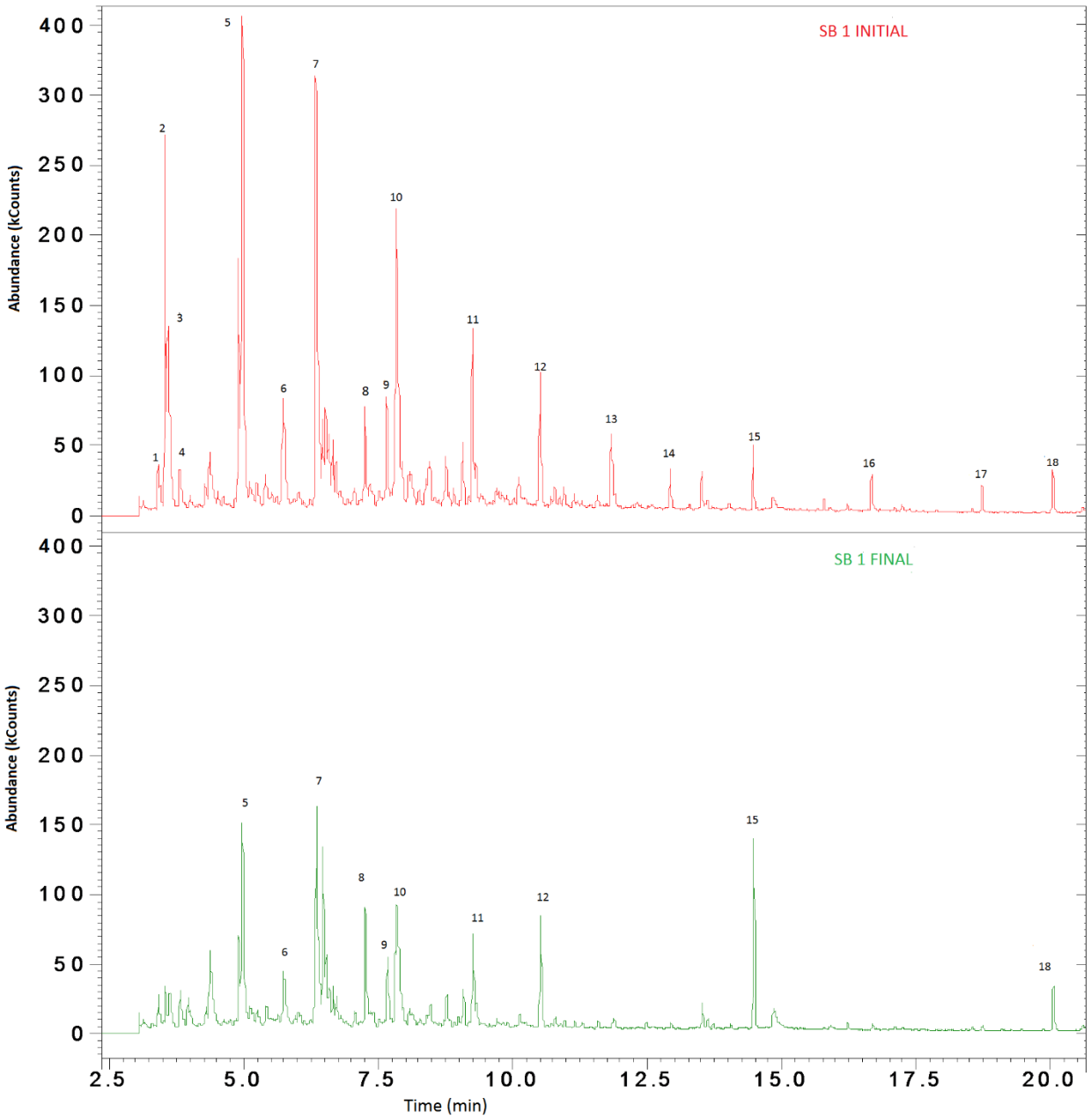

Figure 3. GC/MS chromatograms of the initial and final samples of SB at IC 1.

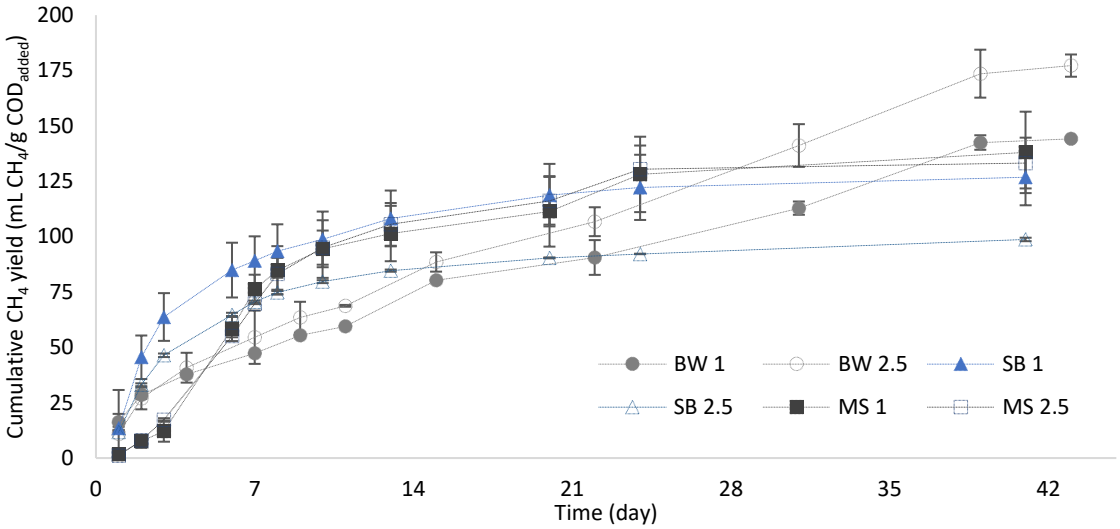

Figure 4. Time-course of cumulative methane yield during the anaerobic digestion of the LFHTC of sewage sludge.